and a truncated MinD mutant protein lacking the membrane targeting sequence (MTS); both MinD mutant proteins are unable to polymerize with MinC. Additionally, the in vitro activity of MinC to prevent GTP-dependent FtsZ pelleting in sedimentation assays is impaired by MinD and ATP, suggesting polymerization with MinD may modulate MinC function. Finally, addition of MinE to MinC-MinD polymers induces concentration-dependent disassembly manner suggesting MinE competes with MinC for binding to MinD. Results suggest a novel nucleotide-dependent conformation of MinD with MinC and provides mechanistic insight into the functional interactions of the Min proteins.

3024-Pos Board B454
Superresolution Investigation of the E. coli Cell Division Ring during Constriction
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The *E. coli* cell division machinery are recruited to midcell by a ring-like structure formed by the highly conserved FtsZ protein. This ''Z-ring'' scaffold can generate a membrane bending force that may be critical for cytokinesis. Several mechanistic models for Z-ring force generation have been presented, but are limited in detail by insufficient data regarding the arrangement of FtsZ filaments within the Z-ring and regarding the role of FtsZ's GTPase activity in constriction progression.
Structural details about the Z-ring have recently become clearer due to advancements in both optical and electron microscopy. Super-resolution fluorescence microscopy and electron cryotomography studies support a loosely bundled arrangement of FtsZ filaments, resulting in both sparse regions and dense clusters within the Z-ring. Super-resolution imaging also revealed that the Z-ring can adopt both a single-ring conformation at midcell and a multiple-ring conformation reminiscent of a tight helix.
We have built upon these previous studies to determine the three-dimensional structure of the *E. coli* Z-ring in super resolution using Photoactivated Localization Microscopy (PALM) and analyze its changes during constriction. We used quantitative analyses of PALM and time-lapse data to assess the plausibility of various FtsZ-centric constriction mechanisms. We found that the cell wall constriction rate is not correlated to Z-ring assembly/disassembly dynamics, density, or GTPase activity. These characterizations help refine and re-define the role of FtsZ in cytokinesis.

3025-Pos Board B455
Bacterial Growth and Shape Regulation by External Compression
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Gram-negative Escherichia coli are rod-shaped and maintain a constant diameter during their exponential growth phase. This shape has been shown to be critical in regulating many fundamental biological functions including nutrient access, cell division, attachment, dispersal, and motility. Here, we experimentally show that mechanical forces regulate growth and shapes of E. coli. By applying precisely controllable compressive forces to growing rod-shaped E. coli, we demonstrate that the cells no longer retain their rod shapes but grow and divide with pancake-like shapes while these deformed cells can recover their shapes in several generations after the forces are removed. Those cells are proved to have comparable growth rate, proliferation rate, DNA replication, and protein synthesis to rod-shaped cells. Furthermore, the growth of compressed cells also helps reveal the biological and mechanical role of MreB in maintaining cell shape.

3026-Pos Board B456
Dissecting the Mechanism of Type VI Secretion System Effector Delivery by Fluorescence Cross-Correlation Microscopy
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The Type Six Secretion System (T6SS) is a bacterial toxin-delivery system targeting bacterial cells which neighbor the donor, promoting recipient cell death. The T6SS is widely conserved among Gram-negative bacteria and may be a central determinant in bacterial fitness in polymicrobial communities of particular relevance to chronic infection. Sequence homology of secretion system components to the T4 bacteriophage tail spike, cryoEM reconstructions of the secretion system and fluorescence imaging are all consistent with a dynamic mechanism of secretion. The complex system, which is composed of at least 15 proteins, forms a puncturing apparatus/delivery system which uses a donor protein filament to puncture the recipient cell wall to deliver protein toxins. Using quantitative imaging analysis of multiple fluorescent fusions, we present a detailed characterization of T6SS system dynamics visualized in single cells in multiple bacterial species, developing a model of T6SS function. We present quantitative measurements of the dynamics of the secretion system - from the assembly to contraction to disassembly - in conjunction with quantitative measures of system function, including recipient cell lysis.

3027-Pos Board B457
Role of Fumarate in the Operation of the Bacterial Flagellar Motor
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Fumarate has previously been identified as a 'switch factor' that can alter the switching frequency and bias of the sense of rotation of the bacterial flagellar motor; however, despite numerous studies during the past fifty years, the molecular mechanism by which fumarate causes these effects could not be understood. We studied the motility of *Salmonella* by bright field and by monitoring rotation in a tethered cell assay. We carried out a detailed computerized analysis of the swimming and rotational behavior in real time under various conditions of external pHs (5.0 $\pm$ 8.0) and added fumarate concentrations (0 $\pm$ 12 mM). Based on our experimental results, we make the novel proposal that the bacterial flagellar motor contains access channels permeable to the fumarate monoanion, and that it catalyzes the overall electroneutral transport of fumarate and protons. We further postulate that each ionic species provides ~50% of the total energy for rotation of the bacterial flagellar motor. These predictions are applicable to both the normal (say CW) mode of motor rotation as well as to the so-called ''switched'' (CCW) sense of rotation, depending on the direction of translocation of the ions. Hence previous proposals of fumarate as a 'switch factor', are false, or at best incomplete, because fumarate participates in the rotation of the flagellar motor in both normal and switched modes of operation. The new hypothesis extends to bacterial motility the essential and vital role played by dicarboxylic acid anions in oxidative phosphorylation and photosynthesis according to Nath's torsional mechanism of energy transduction and ATP synthesis (Nath, S., Beyond the Chemiosmotic Theory, *Journal of Bioenergetics and Biomembranes* 2010,**42**, 301-309).

3028-Pos Board B458
A GTPase Deficient FtsZ Mutant Assembles Inefficiently and Impairs Cytokinesis in Bacillus Subtilis Cells
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FtsZ polymerizes to form a dynamic Z-ring at the mid-cell position and orchestrates the bacterial cell division. The perturbation of Z-ring has been found to be lethal to bacteria. Plumbagin and SB-RA-2001 were found to inhibit bacterial cell proliferation by targeting FtsZ. Docking analyses indicated that both plumbagin and SB-RA-2001 bind to the cleft region between H7 helix and C-terminal domain of the *Bs*FtsZ. Arg191 (R191) was identified as the common residue involved in H-bonding with these two compounds. To examine the importance of R191residue in the assembly of FtsZ, we constructed R191A-*Bs*FtsZ. The secondary structure of the mutated FtsZ (R191A-*Bs*FtsZ) was similar to that of WT-*Bs*FtsZ. The mutation (R191A) strongly diminished the GTPase activity of FtsZ. R191A-*Bs*FtsZ exhibited less polymerization ability as compared to WT-*Bs*FtsZ. It could also poison the assembly of WT-*Bs*FtsZ in a concentration dependent manner. Moreover, R191A-*Bs*FtsZ formed polymers of a different morphology than that of WT-*Bs*FtsZ. *In-silico* study revealed that R191A *Bs*FtsZ interacts with WT-BsFtsZ and forms a curved dimer while two WT-*Bs*FtsZ monomers form a linear dimer. Further, we observed that when R191A-*Bs*FtsZ was transformed into *B. subtilis* PL2084 strain, the *B. subtilis* cells became filamentous indicating that the mutation had a strong adverse effect on the division of *B. subtilis* cells. The results suggested that the GTPase activity of FtsZ plays an important role in Z-ring formation. The study also suggested that designing antimicrobials targeting the Arg191 residue might help to curb the division process.

3029-Pos Board B459
Bacterial Motility Measured by a Miniature Chamber for High-Pressure Microscopy
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Hydrostatic pressure is one of the physical stimuli that characterize the environment of living matter. Many microorganisms thrive under high pressure and may even physically or geochemically require this extreme environmental condition. In contrast, application of pressure is detrimental to most life on Earth; especially to living organisms under ambient pressure conditions. To study the mechanism of how living things adapt to high-pressure conditions, it is necessary to monitor directly the organism of interest under various pressure

conditions. Here, we report a miniature chamber for high-pressure microscopy [1]. The chamber was equipped with a built-in separator, in which water pressure was properly transduced to that of the sample solution. The apparatus developed could apply pressure up to 150 MPa, and enabled us to acquire bright-field and epifluorescence images at various pressures and temperatures. We demonstrated that the application of pressure acted directly and reversibly on the swimming motility of *Escherichia coli cells*. The present technique should be applicable to a wide range of dynamic biological processes that depend on applied pressures [2, 3].

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[2] Nishiyama M. *et al.* 2013. High Hydrostatic Pressure Induces Counterclockwise to Clockwise Reversals of the *Escherichia coli* Flagellar Motor. *J. Bactetiol.***195**: 1809-1814.
[3] Okuno D. *et al.*, 2013. Single-Molecule Analysis of the Rotation of F_1-ATPase under High Hydrostatic Pressure. *Biophys. J.***105**:1635-1642.

3030-Pos Board B460

Bacterial Flagellar Switching: Hidden Markov Steps Revealed

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Here we describe a Markov chain that accounts for hidden steps in the switching mechanism of bacterial flagellar rotation. The bacterial flagellar motor apparatus switches between counter clockwise rotation (CCW) and clockwise rotation (CW) by a process that requires interactions between ligand-bound switch subunits of the rotor and motor units of the stator. If the ligand, CheY, binding to switch subunits shifts equilibrium between CCW and CW, i.e. simplest proposed mechanism, the intervals between switches, dwell times, should be a random variable of an exponential distribution. Because dwell times are actually gamma distributed, the switching mechanism has evidence for multiple Poisson steps, which is characteristic of a Markov process. We are proposing that the hidden steps of the switching mechanism are intermediates of the principle thermodynamic states, CCW and CW, generated by rotation. At steady-state, the intermediates factor out of the derived state function, but both the thermodynamic states and intermediates appear as statistical states of a Markov chain. The state function determines the bias and the conditional probabilities for all steps of the Markov chain except when rotation separates a switch subunit, which must have unit probability. To obtain a continuous time random walk, we applied the rotation rate to all conditional and unit probabilities of the discrete Markov chain. Published dwell time distributions were fit best by simulations that depend on bias alone regardless of the number of motor units operating on a rotor. Because the bias in our model depends on the probability that all motor units of a rotor interact with switch subunits that are ligand bound, each motor unit contributes less to the free energy change required for a given bias as motor units increase. Yet the probability of a switch remains constant.

3031-Pos Board B461

Mechanical Stress Changes the Movements and Organization of Biofilm-Associated Bacteria

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Historically, studies analyzing collective movements, biofilm formation, and the emergence of pathogenicity in bacteria have focused on chemical signals that elicit changes in cell behavior, while responses to physical cues have only recently begun to garner attention. In contrast, the effects of changes in mechanical properties of the environment on eukaryotic cells have been studied extensively. It is known, for instance, that substrate stiffness can guide the migration of epithelial and endothelial cells and has profound effects on cell shape and division. Our research aims to examine how bacteria and bacterial colonies respond to changes in the physical properties of their environment (mechanical stress, stiffness, and surface topography) and to understand the molecular mechanisms behind the response. Previous work showed that the Gram-negative, biofilm-forming bacterium Myxococcus xanthus responds to tension and compression of its substrate's surface by forming elliptical colonies that expand most rapidly perpendicular to the axis of compression. This behavior, dubbed "elasticotaxis", was initially hypothesized to be important for locating food sources. By combining physics-based modelling of the stresses in compressed substrates with experimental data showing the corresponding change in shape of M. xanthus colonies, we have established a linear correlation between the mechanical stress in the substrate and the degree of the elasticotaxis response. To identify the physical changes in the substrate that elicit the elasticotaxis response, we are investigating whether compression of the agar substrate leads to changes in topography, such as wrinkling, or to changes in material properties, such as polymer alignment. Recently, we found that elasticotaxis is not unique to M. xanthus; our preliminary results suggest many bacteria exhibit this behavior. Accordingly, we are examining whether elasticotaxis is common to both gliding bacteria and swarming bacteria and how it may affect biofilm formation.

3032-Pos Board B462

Atomic Force Microscope Spectroscopy: Progress toward Antibiotic Resistance and Biofilm Studies

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The emerging field of live cell nanoscopy and force spectroscopy could revolutionize the way biologists explore the living cell at a molecular resolution. Atomic Force Microscope (AFM) and force spectroscopy analysis have been used to directly measure reversible physiochemical and specific binding interactions between cells. Stickiness is important biofilm formation stage that could also be measured at nN level. A significant source of foodborne illness results from biofilms. These are caused by microorganisms that attach to surfaces and grow as highly organized multicellular communities. This study examines the impact of silver resistance on bacterial adhesion and its viscoelastic formation. We present the first set of data that evaluates the elastic deformation of a bacterial cell surface upon evolution of silver resistance in E. coli MG1655 using AFM compared to controls in 200 generations. The adhesion stickiness and stiffness mean of the treated (evolved Ag resistant) and non-treated samples were tested at nN level. The evolved samples had a significantly ($P<0.05$) higher stickiness ratio and value (from 0.01 ± 0.04 nN to 0.06 ± 0.02 nN) compared to the controls (non-resistant) strains (from0.01 ± 0.02 nN to0.04 ± 0.02 nN). The highest difference of adhesion force happened on Generation 100. According to images of the bacteria in different generations, we can see some major phenotype changes on the appearance at generation 100. The MIC data for the non-evolved strains of E. coli MG 1655 through 200 generations were also significantly lower 38.58 ± 10.09 mg/l compare to evolved strains 272.25 ± 153.94 mg/l ($p<0.01$). The experiment demonstrates important features of phenotype modulation resulting from the evolution of Ag resistance that will be further studied by this group.

3033-Pos Board B463

Depletion-Mediated Pattern Formation in a Growing Bacterial Colony

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Secretion of extracellular polymeric substances into growth medium of bacteria is the hallmark of forming biofilm-like structures. The morphological property of such systems might depend upon the physical interactions of cells with extracellular polymeric substances(EPS). We have studied self-organization of nonmotile rod-shaped bacterial cells growing on solid substrate in presence of self-producing EPS, secreted into the growth medium in expanding colony. In our individual-based simulation model of bacterial cells and EPS, all the particles interact mechanically via repulsive forces by pushing each other away as bacterial cells grow and divide consuming diusing nutrient and produce EPS. We show that mechanical interactions control the collective behavior of the system, particularly, we show that the presence of nonadsorbing EPS leads spontaneous aggregation of bacterial cells by depletion attraction and generates phase separated patterns in a nonequilibrium growing colony. This generic mechanism powered by entropic forces could explain one of the possible ways to spontaneous aggregated structure formation and spatial heterogeneity in a biofilm.

3034-Pos Board B464

Bacterial Chemotactic Tumble Angles Reduce Backtracking and Maximize Information Gathering

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Chemotaxing bacteria gather information from the environment and use that to control the balance between runs and tumbles in order achieve a biased motion toward the source of a chemoattractant. We have examined the role of the tumble angle on how effectively gradients are coupled into a bacterium's trajectory. Chemotaxis was simulated using the ZBP program, and the average tumble angle varied from 0 to 180 degrees in the presence and absence of the normal angle variance and/or rotational diffusion. 100,000 step (0.1 μm/step) trajectories from these simulations where analysed using the k-space information